Requirement for Leukotriene B_4 Receptor 1 in Allergen-induced Airway Hyperresponsiveness

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Rationale: **Leukotriene B_4 (LTB_4) is a rapidly synthesized, early leukocyte chemoattractant that signals via its cell surface receptor, leukotriene B_4 receptor 1 (BLT1), to attract and activate leukocytes during inflammation. A role for the LTB_4–BLT1 pathway in allergen-induced airway hyperresponsiveness and inflammation is not well defined.** ***Objectives:*** **To define the role of the LTB_4 receptor (BLT1) in the development of airway inflammation and altered airway function.** ***Methods:*** **BLT1-deficient ($BLT1^{-/-}$) mice and wild-type mice were sensitized to ovalbumin by intraperitoneal injection and then challenged with ovalbumin via the airways. Airway responsiveness to inhaled methacholine, bronchoalveolar lavage fluid cell composition and cytokine levels, and lung inflammation and goblet cell hyperplasia were assessed.** ***Results:*** **Compared with wild-type mice, $BLT1^{-/-}$ mice developed significantly lower airway responsiveness to inhaled methacholine, lower goblet cell hyperplasia in the airways, and decreased interleukin (IL)-13 production both *in vivo*, in the bronchoalveolar lavage fluid, and *in vitro*, after antigen stimulation of lung cells in culture. Intracellular cytokine staining of lung cells revealed that bronchoalveolar lavage IL-13 levels and numbers of IL-13^+/$CD4^+$ and IL-13^+/$CD8^+$ T cells were also reduced in $BLT1^{-/-}$ mice. Reconstitution of sensitized and challenged $BLT1^{-/-}$ mice with allergen-sensitized $BLT1^{+/+}$ T cells fully restored the development of airway hyperresponsiveness. In contrast, transfer of naive T cells failed to do so.** ***Conclusion:*** **These data suggest that BLT1 expression on primed T cells is required for the full development of airway hyperresponsiveness, which appears to be associated with IL-13 production in these cells.**

Keywords: airway responsiveness; cytokines; lipid mediators; lung inflammation; T cells

Airway hyperresponsiveness (AHR) is the result of complex pathophysiologic changes in the airways and is a predominant feature of allergic asthma. Allergen-specific memory T cells and antibody are thought to play a central role in the development of this response (1–3). Studies in both humans and rodents have indicated that in addition to $CD4^+$ T cells, $CD8^+$ T cells may play an important role in the development of allergic airway responses (4–9).

Leukotriene B_4 (LTB_4) is a potent lipid inflammatory mediator derived from membrane phospholipids by sequential actions of cytosolic phospholipase A_2, 5-lipoxygenase, and leukotriene A_4 hydrolase (10, 11). LTB_4 is a chemoattractant for leukocytes, including neutrophils, macrophages, monocytes, and eosinophils (12–15). LTB_4 activates leukocytes through a G protein–coupled cell surface receptor, leukotriene B_4 receptor 1 (BLT1) (16, 17). BLT1 has been shown to be expressed on effector T cells and the LTB_4–BLT1 pathway may be important in effector T-cell movement to sites of acute inflammation (18, 19).

The present study investigated the role of BLT1 in the development of allergen-induced AHR and airway inflammation, using mice that do not express this receptor. $BLT1^{-/-}$ mice showed significantly decreased allergen-induced AHR and goblet cell hyperplasia in the airways, which was associated with a reduction in interleukin (IL)-13 production from lung $CD4^+$ and $CD8^+$ T cells. Reconstitution of $BLT1^{-/-}$ mice with antigen-sensitized $BLT1^{+/+}$ T cells fully restored the development of ovalbumin (OVA)-induced AHR, indicating that BLT1 expression on T cells may play an important role in allergen-induced AHR.

METHODS

See the online supplement for details of the methods used in this research.

Animals

Female mice, with a targeted disruption of the LTB_4 receptor ($BLT1^{-/-}$ mice) and backcrossed for seven generations onto a BALB/c genetic background (20), and $BLT1^{+/+}$ mice 8 to 12 weeks of age were used in all experiments.

Sensitization and Airway Challenge

$BLT1^{-/-}$ and $BLT1^{+/+}$ mice were assigned to the following groups: (*1*) nonsensitized but airway challenged with OVA nebulization alone (phosphate-buffered saline [PBS]/OVA group) and (*2*) intraperitoneal sensitization with OVA and OVA airway challenge (OVA/OVA). Mice were sensitized by intraperitoneal injection of 20 μg of OVA (Grade V; Sigma, St. Louis, MO) emulsified in 2.25 mg of alum (Imject Alum; Pierce Biotechnology, Rockford, IL) in a total volume of 100 μl on Days 1 and 14. Mice were subsequently challenged via the airways by inhalation exposure to aerosols of OVA (1% in saline) for 20 minutes on Days 28, 29, and 30. On Day 32, airway function was measured as described below, followed by collection of samples for further analyses.

Assessment of Airway Function

Mice were anesthetized, tracheostomized, and mechanically ventilated, and airway function was assessed as previously described by measuring changes in lung resistance (R_L) and dynamic compliance (Cdyn) in response to increasing doses of inhaled methacholine (21). Data are expressed as percent change from baseline R_L and Cdyn values obtained after inhalation of saline. There were no significant differences in baseline values among the different groups.

Bronchoalveolar Lavage

Shandon Cytospin slides (Thermo Electron, Waltham, MA) were stained with Leukostat (Fisher Diagnostics, Pittsburgh, PA) and differentiated by standard hematologic procedures.

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Measurement of Total and OVA-specific Antibodies

Serum levels of total IgE, OVA-specific IgE, and IgG1 were measured by ELISA as previously described (22).

Histologic Studies

After lavage, lungs were fixed in 10% formalin and processed into paraffin blocks. Tissue sections, 5-μm thick, were stained with hematoxylin and eosin and periodic acid–Schiff (PAS) for identification of mucus-containing cells. The number of mucus-containing cells per millimeter of basement membrane was determined with IPLab 2 software (Scananalytics, Fairfax, VA) for the Macintosh computer, counting six to eight different fields per animal as previously described (22).

Cells containing eosinophilic major basic protein (MBP) were identified by immunohistochemical staining as previously described, using rabbit antimouse MBP (kindly provided by J. J. Lee, Mayo Clinic, Scottsdale, AZ) (22). The number of peribronchial eosinophils in tissues was evaluated as previously described (22).

Cell Preparation and Culture

Spleen and peribronchial lymph node (PBLN) were removed and mononuclear cells (MNCs) were purified by Ficoll-Hypaque gradient centrifugation (Organon Teknika, Durham, NC) as previously described (7). Lung MNCs were isolated as previously described (23) by collagenase digestion. MNCs (4×10^5) were cultured for 24 hours in 96-well round-bottom plates in the presence or absence of OVA (10 μg/ml) as previously described (7).

Measurement of Cytokines

Cytokine levels in the bronchoalveolar lavage (BAL) fluid and cell culture supernatants were measured by ELISA as previously described (22). IFN-γ, IL-4, IL-5 (BD Biosciences Pharmingen, San Diego, CA), and IL-13 (R&D Systems, Minneapolis, MN) ELISAs were performed according to the manufacturer's directions.

Flow Cytometry

Intracytoplasmic cytokine staining for IL-4, IL-5, IFN-γ (BD Biosciences Pharmingen), and IL-13 (R&D Systems) was performed as previously described (7). The number of cytokine-producing $CD3^+$, $CD4^+$, or $CD8^+$ T cells per lung was calculated from the percentage of cytokine-producing cells and the number of $CD3^+$, $CD4^+$, or $CD8^+$ T cells isolated from the lungs.

Purification of T Cells: Donor Cells

Spleens of $BLT1^{+/+}$ mice, which were sensitized twice (Days 1 and 14) with OVA plus alum, were removed 14 days after the last sensitization (Day 28). MNCs were first isolated by Ficoll-Hypaque gradient centrifugation (Organon Teknika). T cells were isolated by nylon wool passage. Spleens of naive $BLT1^{+/+}$ mice were also removed and purified in the same way. To assess purification, cells were incubated with allophycocyanin-conjugated anti-CD3, fluorescein isothiocyanate–conjugated anti-CD4, and phycoerythrin-conjugated antibodies (anti-CD8, anti-CD11c, antimouse pan-natural killer [NK] cells, anti-B220, anti-γδ TCR, or anti-αβ TCR; BD Biosciences Pharmingen), and then analyzed by flow cytometry (FACSCalibur; BD Biosciences Immunocytometry Systems, San Jose, CA).

Adoptive Transfer: Recipient Mice

Recipient $BLT1^{-/-}$ mice were sensitized twice with OVA plus alum on Days 1 and 14. OVA-primed T cells (5×10^6) were administered intravenously via the tail vein to OVA-sensitized $BLT1^{-/-}$ mice, 14 days after the last sensitization (Day 28). After transfer, the mice were exposed to three allergen challenges via the airways on Days 28, 29, and 30. Assays were performed on Day 32.

Statistical Analysis

Values for all measurements are expressed as means ± SEM. Analysis of variance was used to determine the levels of difference between all groups. Comparisons for all pairs were performed by unpaired two-tailed Student *t* test. Significance levels were set at a p value of 0.05.

RESULTS

$BLT1^{-/-}$ Mice Develop Significantly Lower AHR Compared with $BLT1^{+/+}$ Mice after Sensitization and Challenge

We first assessed airway responsiveness to inhaled methacholine in $BLT1^{-/-}$ mice. Intraperitoneal OVA sensitization and airway challenge led to the development of increased AHR in $BLT1^{+/+}$ mice, illustrated by significant increases in RL and decreases in Cdyn, as compared with mice challenged (but nonsensitized) with OVA (Figures 1A and 1B). In contrast, OVA-sensitized and -challenged $BLT1^{-/-}$ mice developed lower increases in RL and decreases in Cdyn compared with sensitized and challenged $BLT1^{+/+}$ mice, but nonetheless the changes were significantly greater than in $BLT1^{-/-}$ mice that were challenged alone.

Lung Inflammation in $BLT1^{-/-}$ Mice and $BLT1^{+/+}$ Mice

The numbers and types of inflammatory cells in BAL fluid from the airways of $BLT1^{-/-}$ and $BLT1^{+/+}$ mice were examined (Figure 2). After sensitization and allergen challenge, the proportion of eosinophils reached up to 50% of total BAL cells in $BLT1^{+/+}$ mice. $BLT1^{-/-}$ mice showed similar increases in eosinophil number in BAL fluid. There were also no statistically significant differences between the two groups in terms of the numbers of total cells, neutrophils, or lymphocytes recovered in the BAL fluid.

Lung histology showed an infiltration of inflammatory cells into the perivascular and, to a lesser extent, peribronchial spaces in both OVA-sensitized and -challenged $BLT1^{-/-}$ and $BLT1^{+/+}$ mice. Both strains of mouse exposed to OVA challenge alone had no signs of inflammation (data not shown). There were no obvious differences between the two mouse strains when sections stained with hematoxylin and eosin were examined. The number of eosinophils in lung tissue was evaluated by immunohistochemistry, that is, staining for major basic protein (MBP). Comparison of the number of major basic protein–positive cells in peribronchial, perivascular, and parenchymal areas of the lungs did not reveal significant differences between sensitized and challenged $BLT1^{+/+}$ and $BLT1^{-/-}$ mice (data not shown).

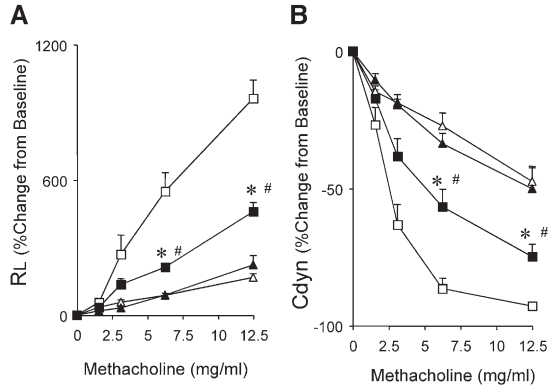

Figure 1. Development of altered airway function in $BLT1^{-/-}$ mice and $BLT1^{+/+}$ mice. Lung resistance (RL; *A*) and dynamic compliance (Cdyn; *B*) values were obtained in response to increasing concentrations of inhaled methacholine as described in METHODS (n = 8 in each group). * Significant difference ($p < 0.01$) between BLT1-deficient mice ($BLT1^{-/-}$ ovalbumin [OVA]/OVA) and BLT1-sufficient mice ($BLT1^{+/+}$ OVA/OVA); # significant difference ($p < 0.05$) between $BLT1^{-/-}$ OVA/OVA versus $BLT1^{-/-}$ phosphate-buffered saline (PBS)/OVA and $BLT1^{+/+}$ PBS/OVA.

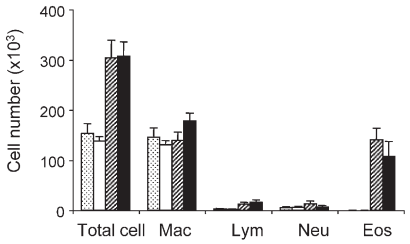

Figure 2. Cellular composition in bronchoalveolar lavage (BAL) fluid. BAL fluid was obtained from the same groups described in the legend to Figure 1. *Dotted bars*, BLT1$^{+/+}$ PBS/OVA; *white bars*, BLT1$^{-/-}$ PBS/OVA; *hatched bars*, BLT1$^{+/+}$ OVA/OVA; *black bars*, BLT1$^{-/-}$ OVA/OVA. Eos = eosinophils; Lym = lymphocytes; Mac = macrophages; Neu = neutrophils.

TABLE 1. CONCENTRATIONS OF TOTAL IgE AND OVALBUMIN-SPECIFIC ANTIBODIES IN THE SERUM OF BLT1$^{+/+}$ AND BLT1$^{-/-}$ MICE

	PBS/OVA		OVA/OVA	
	BLT1$^{+/+}$	BLT1$^{-/-}$	BLT1$^{+/+}$	BLT1$^{-/-}$
Total IgE, ng/ml	139 ± 32	148 ± 45	340 ± 35*	351 ± 42*
OVA-specific IgE, EU/ml†	< 10	< 10	2,755 ± 768*	3,226 ± 716*
OVA-specific IgG1, EU/ml	< 10	< 10	2,167 ± 279*	2,232 ± 320*

Definition of abbreviations: BLT1$^{+/+}$ OVA/OVA = BLT1$^{+/+}$ mice, sensitized and challenged; BLT1$^{-/-}$ OVA/OVA = BLT1$^{-/-}$ mice, sensitized and challenged; BLT1$^{+/+}$ PBS/OVA = BLT1$^{+/+}$ mice, challenged only; BLT1$^{-/-}$ PBS/OVA = BLT1$^{-/-}$ mice, challenged only.

Mice were sensitized and challenged as described in METHODS. Serum levels of immunoglobulins were assessed 48 hours after the last challenge. Mean values ± SEM are given.

* $p < 0.05$, comparing PBS/OVA with OVA/OVA.

† EU/ml, ELISA units/ml.

To assess the degree of goblet cell hyperplasia, tissue sections were stained with PAS (Figure 3). No PAS-positive cells were observed in the airways of nonsensitized but OVA-challenged mice of either strain. However, after OVA sensitization and challenge, BLT1$^{+/+}$ mice developed a significantly higher number of PAS-positive goblet cells in the airways as compared with BLT1$^{-/-}$ mice (cells per millimeter of basement membrane [mean ± SEM]: 50.6 ± 5.8 in BLT1$^{+/+}$ mice vs. 32.3 ± 5.2 in BLT1$^{-/-}$ mice; $p < 0.05$).

OVA-specific Antibody Responses in BLT1$^{-/-}$ Mice and BLT1$^{+/+}$ Mice

Levels of OVA-specific antibodies and total IgE levels in the serum were not significantly different between BLT1$^{+/+}$ mice and BLT1$^{-/-}$ mice after sensitization and challenge with OVA (Table 1). Challenge alone on 3 consecutive days was insufficient to trigger antibody responses in either group of mice.

Cytokine Levels in BAL Fluid

Concentrations of IL-4, IL-5, IL-13, and IFN-γ in BAL fluid were measured by ELISA. OVA sensitization and challenge resulted in significant increases in IL-4 and IL-5 levels and decreases in IFN-γ in both strains of mouse and the results were not significantly different (Figure 4). However, after sensitization and challenge, the levels of IL-13 also increased significantly in both strains of mouse but were significantly lower in the BAL fluid recovered from BLT1$^{-/-}$ mice as compared with BLT1$^{+/+}$ mice.

Numbers of CD3$^+$, CD4$^+$, and CD8$^+$ T Cells in the Lungs and BAL Fluid

We next determined whether BLT1 participates in T-cell trafficking to the lungs and BAL fluid, 48 hours after challenge. Increased numbers of T cells were recovered in the BAL fluid and lungs after OVA challenge from both strains of mouse compared with challenged-only mice, and the distributions of

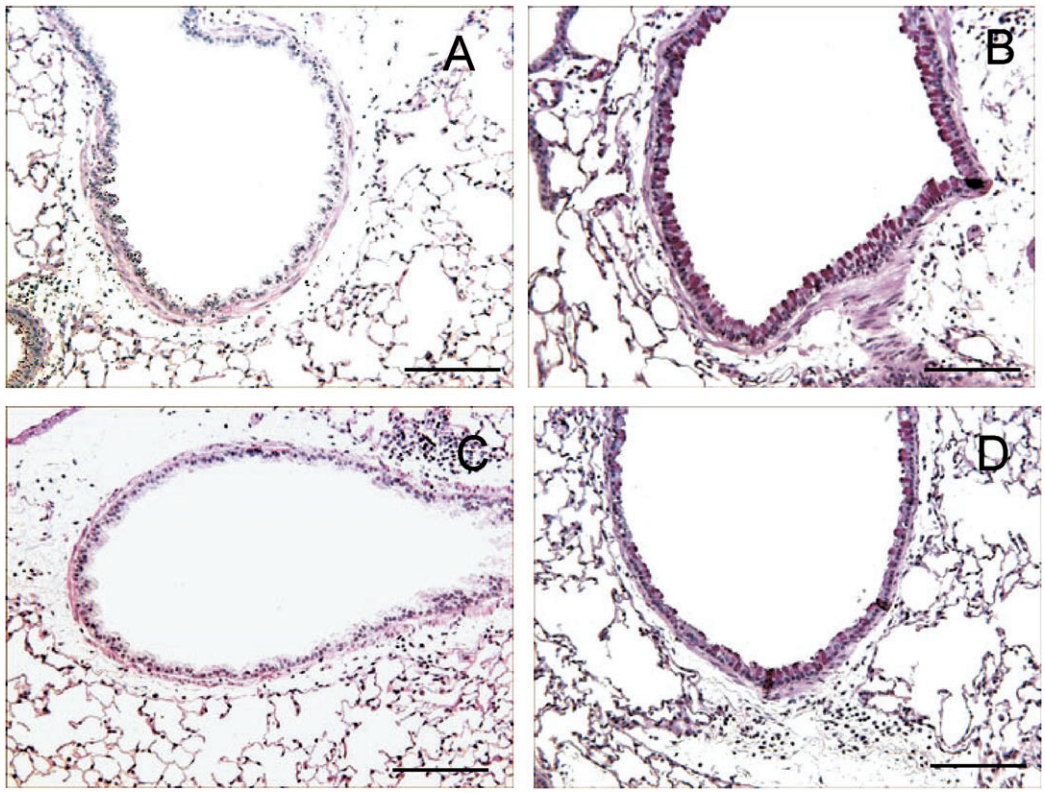

Figure 3. Development of goblet cell hyperplasia in BLT1$^{-/-}$ and BLT1$^{+/+}$ mice. Goblet cell hyperplasia was quantified in periodic acid–Schiff (PAS)-stained sections as described in METHODS. (*A*) Nonsensitized BLT1$^{+/+}$ mice; (*B*) OVA-sensitized and -challenged BLT1$^{+/+}$ mice; (*C*) nonsensitized BLT1$^{-/-}$ mice; (*D*) OVA-sensitized and -challenged BLT1$^{-/-}$ mice. *Scale bars*: 100 μm.

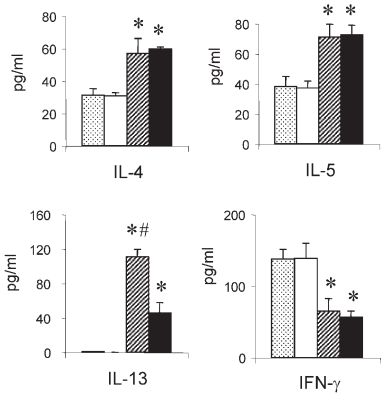

Figure 4. Cytokine levels in BAL fluid. Interleukin (IL)-4, IL-5, IL-13, and IFN-γ levels in BAL fluid from the same groups of mice as in Figure 1 were measured in supernatants by ELISA. *Dotted bars*, BLT1$^{+/+}$ PBS/OVA; *white bars*, BLT1$^{-/-}$ PBS/OVA; *hatched bars*, BLT1$^{+/+}$ OVA/OVA; *black bars*, BLT1$^{-/-}$ OVA/OVA. *Significant difference ($p < 0.05$) between PBS/OVA and OVA/OVA; #significant difference ($p < 0.05$) between BLT1$^{-/-}$ OVA/OVA and BLT1$^{+/+}$ OVA/OVA.

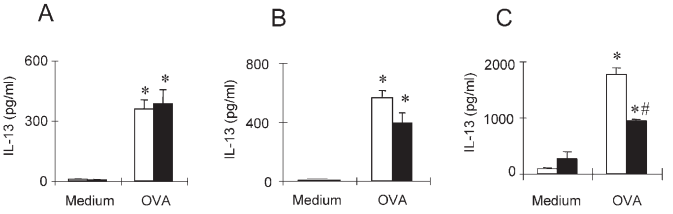

Figure 6. Impaired IL-13 production in lung cells from BLT1$^{-/-}$ mice after sensitization and challenge. IL-13 levels in supernatants from cultured spleen (*A*), peribronchial lymph node (PBLN; *B*), and lung (*C*) MNCs, in the presence or absence of OVA (10 μg/ml), were determined by ELISA. *White bars*, BLT1$^{+/+}$; *black bars*, BLT1$^{-/-}$. *Significant difference ($p < 0.05$) in the presence or absence of OVA; #significant difference ($p < 0.05$) between BLT1$^{-/-}$ and BLT1$^{+/+}$ mice.

CD3$^+$, CD4$^+$, and CD8$^+$ T cells in the lungs or BAL fluid of BLT1$^{-/-}$ mice were not different from those in BLT1$^{+/+}$ mice (Figures 5A and 5B).

In Vitro IL-13 Production from Lung Cells Is Lower in BLT1$^{-/-}$ Mice

To determine whether the difference in IL-13 level observed between the two strains of mouse was due to a difference in antigen-specific T-cell responsiveness, spleen, PBLN, and lung cells were isolated from sensitized and challenged mice and restimulated in culture with OVA. Levels of IL-4, IL-5, IL-13, and IFN-γ were measured in the culture supernatants by ELISA. Consistent with the *in vivo* observations, there were no significant differences between BLT1$^{-/-}$ mice and BLT1$^{+/+}$ mice in terms of IL-4, IL-5, and IFN-γ production after culture with medium alone or with OVA (data not shown). The levels of IL-13 from culture supernatants of spleen and PBLN in sensitized and challenged BLT1$^{-/-}$ mice were similarly not different from those in BLT1$^{+/+}$ mice (Figures 6A and 6B). However, after culture *in vitro* with OVA, lung BLT1$^{-/-}$ cells secreted significantly lower amounts of IL-13 than did lung cells from BLT1$^{+/+}$ mice (Figure 6C).

IL-13 Production from Lung CD4$^+$ and CD8$^+$ T Cells of BLT1$^{-/-}$ Mice Is Lower Compared with BLT1$^{+/+}$ Mice

The two major findings in BLT1$^{-/-}$ mice were attenuation of the AHR response and substantially reduced *in vivo* and *in vitro* production of IL-13. To determine whether the absence of BLT1 affected effector cell function in the lung T cells, we assessed IL-13 production at the single-cell level. The number of IL-13–producing CD4$^+$ and CD8$^+$ T cells from the lungs of sensitized and challenged BLT1$^{-/-}$ mice was significantly lower than in BLT1$^{+/+}$ mice (Figures 7A and 7B). (The anti–IL-13 antibody

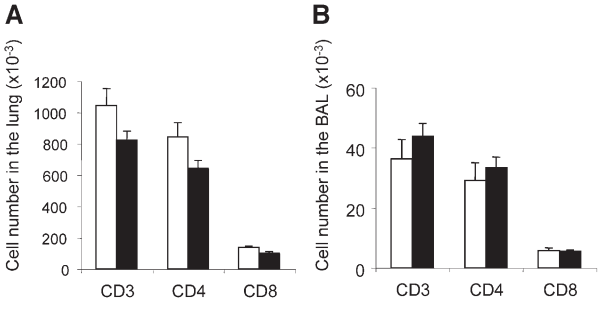

Figure 5. Number of CD3$^+$, CD4$^+$, and CD8$^+$ T cells in lungs and BAL fluid after sensitization and challenge. The number of cells in lungs (*A*) and BAL fluid (*B*) was determined as described in METHODS. *White bars*, BLT1$^{+/+}$; *black bars*, BLT1$^{-/-}$.

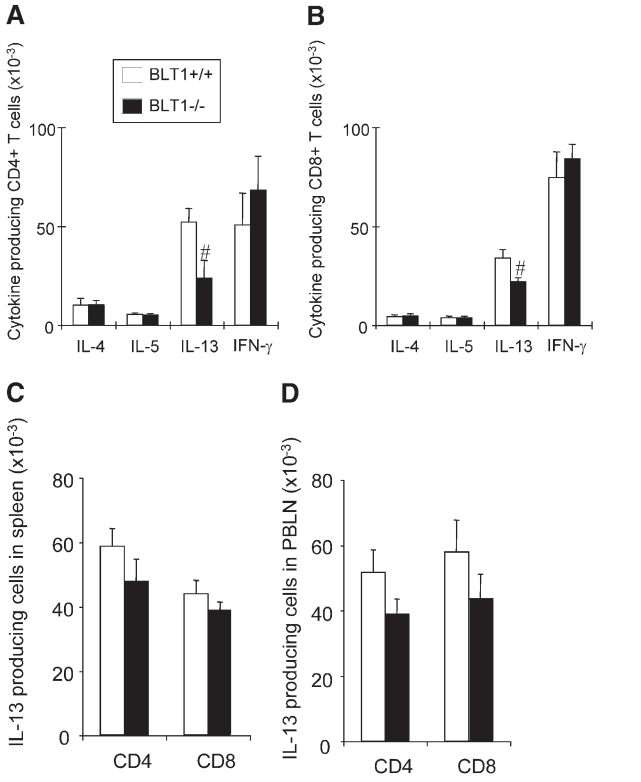

Figure 7. Number of IL-13–producing CD4$^+$ and CD8$^+$ T cells in lungs from BLT1$^{+/+}$ mice was lower compared with BLT1$^{-/-}$ mice after sensitization and challenge with OVA. Number of cytokine-producing CD4$^+$ (*A*) and CD8$^+$ (*B*) T cells in lungs from BLT1$^{+/+}$ and BLT1$^{-/-}$ mice, after sensitization and challenge with OVA, and number of IL-13–producing CD4$^+$ and CD8$^+$ T cells in spleens (*C*) and PBLN (*D*) were determined. Mononuclear cells (MNCs) were isolated and stimulated with phorbol myristate acetate and ionomycin, and then intracellular detection of cytokines was performed as described in METHODS. Number of cytokine-producing CD4$^+$ and CD8$^+$ T cells was determined as described in METHODS. #Significant difference ($p < 0.05$) between BLT1$^{-/-}$ and BLT1$^{+/+}$ mice.

used here was a polyclonal antibody and produced more background staining when compared with monoclonal anti–IL-4, anti–IL-5, and anti–IFN-γ antibodies [7]). In contrast, the numbers of IL-13–producing $CD4^+$ and $CD8^+$ T cells from spleen and PBLN of $BLT1^{-/-}$ mice were not different from those in $BLT1^{+/+}$ mice after sensitization and challenge (Figures 7C and 7D). These results suggested that although overall recruitment of total T cells into the lungs was not influenced by BLT1 expression (Figure 5), effector cell function in lung $CD4^+$ and $CD8^+$ T cells, in particular IL-13 production, was reduced in $BLT1^{-/-}$ mice.

Reconstitution of $BLT1^{-/-}$ Mice

To address whether the absence of BLT1 expression on T cells was responsible for the decreases in AHR, we reconstituted $BLT1^{-/-}$ mice with $BLT1^{+/+}$ T cells. Recipient $BLT1^{-/-}$ mice were sensitized with OVA on Days 1 and 14. On Day 28, 2 hours before OVA challenge, 5×10^6 $BLT1^{+/+}$ T cells were injected intravenously via the tail vein. Donor $BLT1^{+/+}$ mice consisted of two groups: in one group, mice were not sensitized; in the other group, mice were sensitized to OVA on Days 1 and 14 and cells were obtained on Day 28. As assessed by flow cytometry, the cells transferred from OVA-sensitized or naive donor mice were more than 90% ($CD3^+/CD4^+$, 64–67%; $CD3^+/CD8^+$, 25–27%; $CD3^+/\gamma\delta^+$, 0.8–0.9%; $CD3^+$/pan-NK^+, 0.4–0.5%). In both (sensitized/challenged or naive) transferred cell populations, $B220^+$ cells were 5–6%, pan-NK^+ cells were 3–4%, and $CD11c^+$ cells were less than 1%.

After transfer of cells, recipient mice were challenged with OVA on three consecutive days; 48 hours after last challenge, AHR was measured. Figure 8A shows the results of reconstitution with $BLT1^{+/+}$ T cells on airway responsiveness. Reconstitution of the sensitized $BLT1^{-/-}$ mice with antigen-primed $BLT1^{+/+}$ T cells fully restored the development of AHR after challenge, to levels comparable to those seen in sensitized and challenged $BLT1^{+/+}$ mice. In contrast, transfer of naive $BLT1^{+/+}$ T cells failed to restore AHR. Transfer of antigen-primed $BLT1^{+/+}$ T cells to nonsensitized recipients also failed to alter AHR (data not shown). Analysis of cytokine levels in the BAL fluid of recipient mice showed that transfer of OVA-sensitized $BLT1^{+/+}$ T cells (but not of naive $BLT1^{+/+}$ T cells) fully restored IL-13 levels in the BAL fluid (Figure 8B), in parallel with the development of AHR. The levels of other cytokines (IL-4, IL-5, and IFN-γ) were unaffected by either transfer (data not shown). Transfer of antigen-primed $BLT1^{+/+}$ T cells also restored the number of PAS-positive goblet cells in the airways, comparable to wild-type mice, whereas transfer of naive $BLT1^{+/+}$ T cells failed to restore this response (Figure 8C).

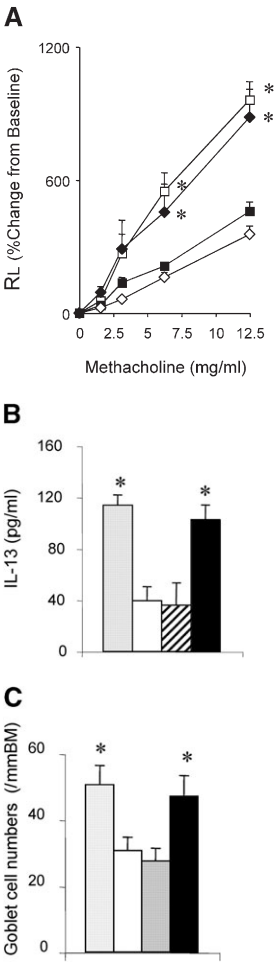

Figure 8. Reconstitution of $BLT1^{-/-}$ mice with antigen-primed $BLT1^{+/+}$ T cells fully restores the development of airway hyperresponsiveness (AHR; *A*), IL-13 levels (*B*), and number of PAS-positive goblet cells in the airways (*C*). OVA-sensitized $BLT1^{-/-}$ mice (recipient mice) received 5×10^6 T cells intravenously via the tail vein, 2 hours before the first airway challenge with aerosolized OVA. Recipient mice comprised two groups: in one group, mice received T cells from spleens of naive $BLT1^{+/+}$ mice (naive donor T group, n = 8); in the other group, mice received T cells from OVA-sensitized $BLT1^{+/+}$ mice (antigen-primed donor T group, n = 8). Sensitized and challenged $BLT1^{+/+}$ and $BLT1^{-/-}$ mice receiving no cells are also shown (n = 8 in each group). AHR was monitored by measuring RL. Cytokine levels were measured in supernatants by ELISA. The number of PAS-positive goblet cells was determined as described in METHODS. (*A*) *White squares*, $BLT1^{+/+}$ OVA/OVA; *black squares*, $BLT1^{-/-}$ OVA/OVA; *white diamonds*, naive donor $BLT1^{+/+}$ T; *black diamonds*, antigen-primed donor $BLT1^{+/+}$ T. (*B, C*) *Dotted bars*, $BLT1^{+/+}$ OVA/OVA; *white bars*, $BLT1^{-/-}$ OVA/OVA; *hatched bars*, naive donor $BLT1^{+/+}$ T; *black bars*, antigen-primed donor $BLT1^{+/+}$ T. *Significant difference ($p < 0.05$) between antigen-primed donor T mice and naive donor T mice. BM = basement membrane.

DISCUSSION

A number of previous studies have shown that LTB_4 may play a central role in the early recruitment of leukocytes to inflamed tissues (12–19). However, its role in the development of AHR and inflammation has not been well defined. Here, we show that the expression of BLT1 can play an important role in the full development of allergen-induced AHR. Using $BLT1^{-/-}$ mice with a targeted disruption of the receptor, allergen-induced AHR was significantly reduced compared with $BLT1^{+/+}$ mice. The number of BAL fluid eosinophils was similar in both strains of mouse, as were serum levels of antigen-specific IgE and IgG1. *In vivo* as well as *in vitro* IL-13 production from lung MNCs was significantly reduced in the deficient mice after sensitization and challenge, suggesting that the BLT1 contribution to the development of AHR may be linked, at least in part, to IL-13 production.

The mechanisms by which BLT1 mediates AHR remain incompletely understood. BLT1 expression on $CD4^+$ and $CD8^+$ T cells may be critical in mediating effector function of lung T cells, especially a subset committed to IL-13 production. IL-13 levels in the BAL fluid of sensitized and challenged $BLT1^{-/-}$ mice were lower, the numbers of $IL\text{-}13^+/CD4^+$ and $IL\text{-}13^+/CD8^+$ T cells in the lungs were also significantly lower than in $BLT1^{+/+}$ mice, and *in vitro* IL-13 production by lung $BLT1^{-/-}$ T cells was lower than in $BLT1^{+/+}$ T cells. After transfer of antigen-primed $BLT1^{+/+}$ T cells, but not of naive $BLT1^{+/+}$ T cells, the development of AHR and levels of IL-13 were fully restored in the $BLT1^{-/-}$ mice. Turner and coworkers (24) have reported that CP-105696, an antagonist of the LTB_4 receptor, suppressed AHR in a primate model, consistent with the present data, although administration of an antagonist (Lγ293111) to a small number of subjects with allergic asthma did not affect the early- or late-phase response to allergen challenge (25). Several previous studies have also shown that LTB_4 may be important for cytokine secretion from T cells *in vitro* (26–30).

It has been shown that BLT1 is expressed on *in vitro*-generated effector $CD4^+$ T cells (19) and $CD8^+$ T cells (18), and that the LTB_4–BLT1 pathway may mediate early recruitment of T cells to the airway (BAL fluid) (19). However, after sensitization to OVA plus alum followed by three consecutive OVA challenges, no differences in number of total T cells in the lungs or BAL fluid could be found between $BLT1^{-/-}$ and wild-type mice (19). This is similar to our findings, in which lung $CD4^+$ and $CD8^+$ T-cell numbers were the same in sensitized and challenged

$BLT1^{+/+}$ and $BLT1^{-/-}$ mice. However, T cells detected in the lungs of the two strains of mouse could be functionally distinguished when numbers of IL–13-producing $CD4^+$ or $CD8^+$ T cells were compared in $BLT1^{+/+}$ and $BLT1^{-/-}$ animals. This functional distinction, based on IL-13, extends previous findings in which sensitized and challenged $CD8^{-/-}$ mice served as recipients. When reconstituted with *in vitro*–generated effector $CD8^+$ T cells (T_{EFF}) or *in vivo*–primed $CD8^+$ T cells from either $BLT1^{+/+}$ or $BLT1^{-/-}$ mice, we found that recruitment of antigen-specific effector $CD8^+$ T cells was indeed dependent on BLT1 expression, as was normalization of IL-13 levels (31). Together, these data suggest that although an increased number of T cells can be detected in the lungs of $BLT1^{-/-}$ mice after sensitization and challenge, the number of IL-13–producing, antigen-specific effector $CD8^+$ (or $CD4^+$) T cells that migrate to the lungs may be limited in the absence of BLT1 expression. Equivalent numbers of IL-13–producing $CD4^+$ and $CD8^+$ T cells were present in the spleens and PBLN of $BLT1^{+/+}$ and $BLT1^{-/-}$ mice. However, the numbers of IL-13–producing $CD4^+$ and $CD8^+$ T cells in the lungs were significantly lower in $BLT1^{-/-}$ mice, suggesting that BLT1 expression may contribute more to the recruitment of IL-13–producing cells to sites of allergic inflammation than to the generation of IL-13–producing cells *in vivo*. Cumulatively, these data identify a critical role for the LTB_4–BLT1 pathway in effector $CD4^+$ and $CD8^+$ T-cell accumulation in the lungs, and in particular, subsets of these cells capable of IL-13 production and enhancing the full development of AHR after challenge of sensitized mice.

BLT1 is a high-affinity receptor for LTB_4 (16), which is expressed mainly on leukocytes (14–16). Several types of leukocytes produce IL-13, including NK cells, NK T cells, and mast cells (32–34). It has also been shown that CD4 is expressed on non-T cells (35). There is a possibility that such cell types secret IL-13 after receiving BLT1 signals. In addition, $CD4^+$ and $CD8^+$ T cells may not only produce IL-13 but also could amplify IL-13 release from these cells.

BLT1 may also be expressed on eosinophils (14–16). Eosinophils show a dose-dependent chemotactic response to LTB_4 and LTB_4 receptor mRNA is upregulated in the airways or lungs of mice after repeated inhalation of *Aspergillus fumigatus* (14). Eosinophilic inflammation in the lungs of $BLT1^{-/-}$ mice was not impaired after OVA sensitization and challenge in the present study or other studies (19), suggesting that recruitment of eosinophils to the lungs is independent of BLT1, at least in these models. *In vivo* as well as *in vitro*, IL-5 production was not impaired in $BLT1^{-/-}$ mice. IL-5 has been clearly defined as a critical factor needed for the differentiation and activation of eosinophils (36) as well as eosinophil recruitment to the lungs. The present data also suggest that BLT1 expression on eosinophils is not essential for development of AHR as transfer of $BLT1^{+/+}$ T cells was able to restore AHR in the absence of BLT1 expression on recipient eosinophils.

After sensitization and challenge, $BLT1^{-/-}$ mice showed a lower degree of goblet cell hyperplasia, compared with $BLT1^{+/+}$ mice. IL-13 has been reported to play a central role in the development of goblet cell hyperplasia (37–39), associated with the induction of *MUC-5* gene expression in epithelial cells (40). Although present to some degree in the deficient mice, our data suggest that the LTB_4–BLT1 pathway does contribute to the full development of goblet cell hyperplasia through the increased accumulation of IL-13–producing $CD4^+$ and $CD8^+$ T cells in the lungs. IL-13 may also be required for the full development of AHR (39, 41, 42). Interestingly, airway responses induced by recombinant IL-13 may also require an intact LTB_4 pathway *in vivo* (43), suggesting that the LTB_4 pathway may also be involved in these IL-13–induced and –dependent events. Because effector T cells in the lungs are a source of IL-13 after allergen challenge, this release of IL-13 may further activate LTB_4 production in the lungs and serve to amplify or enhance the accumulation and activation of effector $CD4^+$ and $CD8^+$ T cells. Our data indicate that the link between BLT1 expression and IL-13 production and a possible amplification loop (LTB_4–BLT1–IL-13) may be critical to the development of allergen-induced AHR and goblet cell hyperplasia in the airways. LTB_4, acting through BLT1, has been shown to be involved in such amplification loops in other conditions. Similar amplification loops involving LTB_4, BLT1, and endogenous monocyte chemoattractant protein 1 (MCP-1/CCL2) production were shown to be important in murine models of acute septic peritonitis (44) and in atherosclerosis (45).

In the absence of BLT1 expression, development of AHR was impaired, but not completely abrogated. IL-13 production from effector T cells in the lungs of $BLT1^{-/-}$ mice was significantly reduced, whereas the production of other cytokines, IL-4, IL-5, and IFN-γ, was not altered. Enhancement of helper T-cell type 2 recruitment into the lungs has been shown to require the expression of parenchymal signal transducer and activator-6 (STAT-6)–inducible chemokines (46). Although the activation of certain effector functions (or subsets) of T cells, especially IL-13 production, may be dependent on the LTB_4–BLT1 pathway, these STAT-6–dependent chemokines may also contribute to recruitment and activation of T-cell effector function in the lungs. Whether this reflects selective T-cell subset effects is under investigation. BLT2 is a low-affinity receptor for LTB_4 (47), and may play some role in mediating LTB_4 function, perhaps even compensating to some degree for the absence of BLT1. The role of this low-affinity receptor for LTB_4 in allergen-induced airway responses remains to be defined.

In summary, we have identified an important role for BLT1 expression on antigen-primed T cells in the development of allergen-induced AHR. In the absence of BLT1, the reduction in AHR is associated with a significant reduction in the number of lung $CD4^+$ and $CD8^+$ T cells producing IL-13 and a resultant decrease in the number of mucus-containing goblet cells. The cumulative data indicate that control of the LTB_4–BLT1 pathway should provide novel therapeutic strategies for the treatment of asthma.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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